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The effect of chronic kidney disease on the urine proteome in the domestic cat (*Felis catus*) / Ferlizza, E.; Campos, A.; Neagu, A.; Cuoghi, Aurora; Bellei, Elisa; Monari, Emanuela; Dondi, F.; Almeida, A. M.; Isani, G..  
- In: THE VETERINARY JOURNAL. - ISSN 1090-0233. - STAMPA. - 204:1(2015), pp. 73-81.  
[10.1016/j.tvjl.2015.01.023]

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24/11/2024 11:18

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1 **The effect of chronic kidney disease on the urine proteome in the domestic cat (*Felis catus*)**

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24 **Abstract**

25           Chronic kidney disease (CKD) is a major cause of mortality in cats, but sensitive and specific  
26 biomarkers for early prediction and monitoring of CKD are currently lacking. The present study aimed  
27 to apply proteomic techniques to map the urine proteome of the healthy cat and compare it with the  
28 proteome of cats with CKD. Urine samples were collected by cystocentesis from 23 healthy young cats  
29 and 17 cats with CKD. One-dimensional sodium-dodecyl-sulfate polyacrylamide gel electrophoresis  
30 (1D-SDS-PAGE) was conducted on 4-12% gels. Two-dimensional electrophoresis (2DE) was applied  
31 to pooled urine samples from healthy cats ( $n = 4$ ) and from cats with CKD ( $n = 4$ ). Sixteen protein  
32 bands and 36 spots were cut, trypsin-digested and identified by mass spectrometry.

33

34           1D-SDS-PAGE yielded an overall view of the protein profile and the separation of  $32 \pm 6$  protein  
35 bands in the urine of healthy cats, while CKD cats showed significantly fewer bands ( $P < 0.01$ ). 2-DE  
36 was essential in fractionation of the complex urine proteome, producing a reference map that included  
37 20 proteins. Cauxin was the most abundant protein in urine of healthy cats. Several protease inhibitors  
38 and transport proteins that derive from plasma were also identified, including alpha-2-macroglobulin,  
39 albumin, transferrin, haemopexin and haptoglobin. There was differential expression of 27 spots  
40 between healthy and CKD samples ( $P < 0.05$ ) and 13 proteins were unambiguously identified. In  
41 particular, increased expression of retinol-binding protein, cystatin M and apolipoprotein-H associated  
42 with decreased expression of uromodulin and cauxin confirmed tubular damage in CKD cats  
43 suggesting that these proteins are candidate biomarkers.

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46

47 *Keywords:* Biomarkers; Cat; Nephropathy; Proteinuria; Electrophoresis

48

## 49 **Introduction**

50           Chronic Kidney Disease (CKD) is the most common renal disease of cats. Its prevalence is  
51 estimated at 1-3% in the general feline population, reaching 50% in geriatric cats (Polzin, 2011;  
52 Bartges, 2012). Most cats with CKD caused by chronic tubulointerstitial nephritis have insignificant  
53 proteinuria (urinary protein to creatinine ratio, UPC < 0.2). However, the minority of cats, particularly  
54 with advanced CKD, could be borderline (UPC 0.2-0.4) or overtly proteinuric (UPC > 0.4), due to a  
55 more severe tubular and glomerular involvement. It is well known that proteinuria itself could promote  
56 further renal damage and CKD progression. However, the mechanism by which these excess proteins  
57 induce renal injury is still not entirely understood (Bartges, 2012).

58

59           Sensitive and specific biomarkers for early prediction and monitoring of CKD in cats are  
60 currently lacking. Quantitative methods for the detection of proteinuria, (urinary protein and urinary  
61 albumin to creatinine ratios; UPC and UAC, respectively) are used to evaluate the severity of renal  
62 involvement but offer no information on its aetiology or on composition of the urine proteome (Tesch,  
63 2010). In addition, cauxin, a 70 kDa protein secreted physiologically by the tubule in cats, can interfere  
64 with the assessment of proteinuria (Mischke, 2011; Miyazaki et al., 2011).

65

66           Urine is considered an ideal source of clinical biomarkers as it can be obtained repeatedly and  
67 noninvasively in sufficient amounts. High-resolution electrophoresis coupled with mass spectrometry  
68 (MS) allows fractionation and identification of the complex urine proteome and can therefore provide  
69 important information not only on kidney function but also on general health status. Over the last few  
70 years, large-scale proteomics have been extensively applied in human medicine, first to define the  
71 protein urine map and then to search for novel biomarkers of pathologies, including CKD (Candiano et  
72 al., 2010; He et al., 2012). In veterinary medicine, the application of proteomics techniques is still

73 limited, but recently there have been significant efforts to study the urine proteome in dogs (Nabity et  
74 al., 2011; Schaefer et al., 2011; Brandt et al., 2014; Miller et al., 2014), and in cats to a lesser extent  
75 (Lemberger et al., 2011; Jepson et al., 2013). The many applications of proteomics have been recently  
76 reviewed (Almeida et al., 2015).

77

78 The aim of the present work was to produce a comprehensive characterization of the urine  
79 proteome of the healthy cats (*Felis catus*) and to compare it with the proteome in CKD patients.  
80 Ultimately we aimed to identify putative biomarkers of nephropathy to be used for detection of CKD or  
81 other renal diseases.

82

### 83 **Material and methods**

#### 84 *Animal selection, sample collection and preparation*

85 The study used privately owned cats divided into two experimental groups. The healthy group  
86 was comprised of entire cats presented to a veterinary teaching hospital for neutering. Only animals  
87 considered healthy on the basis of history and physical examination and with no history of urinary tract  
88 disease were included. The diseased group was comprised of cats with CKD diagnosed on the basis of  
89 history, clinical signs, and clinicopathological and imaging results, according to Bartges (2012). Cats  
90 had to have (1) clinical findings of CKD, (2) persistent pathological renal proteinuria based on the  
91 urine protein to creatinine ratio, assessed and confirmed over a two-month period (UPC >0.2), and (3) a  
92 serum creatinine concentration  $\geq 1.60$  mg/dL and urine specific gravity (USG) <1.035.

93

94 CKD cats were staged according to the International Renal Interest Society (IRIS<sup>1</sup>) CKD  
95 guidelines. Upon arrival, all cats were subjected to a physical examination and routine laboratory tests,

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<sup>1</sup> See: <http://www.iris-kidney.com/guidelines/>

96 including complete blood count, serum chemistry and complete urinalysis with UPC and urine culture.  
97 Five millilitres of urine were collected from each animal by ultrasound-guided cystocentesis. After  
98 centrifugation at 1,500 g for 10 min, supernatants were immediately stored at -80 °C.

99

#### 100 *Urine protein to creatinine ratio*

101 Urine total proteins and creatinine were determined using commercial kits (Urinary/CSF  
102 Protein, OSR6170, and Creatinine OSR6178, Olympus/Beckman Coulter) on an automated chemistry  
103 analyzer (AU 400, Olympus/Beckman Coulter). The UPC was calculated using the following formula:

104

105  $UPC = \text{urine protein (mg/dL)} / \text{urine creatinine (mg/dL)}$ .

106

#### 107 *One-dimensional gel electrophoresis (1D-SDS-PAGE)*

108 Urine proteins were separated using the electrophoresis NuPAGE system (Thermo Fisher  
109 Scientific) on 4-12% polyacrylamide gel in 2-(*N*-morpholino) ethanesulfonic acid buffer with sodium-  
110 dodecyl-sulfate (SDS) (Thermo Fisher Scientific). Two micrograms of protein for each sample were  
111 loaded. The gels were stained with SilverQuest (Thermo Fisher Scientific). After staining, each gel was  
112 digitalized and its pherogram was obtained using GelAnalyzer 2010 software<sup>2</sup>.

113

114 To evaluate differences between genders, two pools were prepared by collecting and mixing 20  
115 µg of proteins from each healthy male ( $n = 8$ ) and female ( $n = 15$ ) sample. The pools were concentrated  
116 by Vivaspin500 spin columns (Sartorius Stedim Biotech) with a molecular weight (MW) cut-off of 3  
117 kDa and separated by 1D-SDS-PAGE with the protocol reported above, with the exception of 3-(*N*-

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<sup>2</sup> See: <http://www.gelanalyzer.com/>

118 morpholino)propanesulfonic acid buffer and Coomassie blue staining (PageBlu protein staining  
119 solution; Thermo Fisher Scientific) compatible with mass spectrometry analysis.

120

### 121 *Two-dimensional gel electrophoresis (2-DE)*

122 Urine samples from four healthy and four CKD cats were selected for 2-DE. To concentrate and  
123 desalt samples, 150 µg of protein for each sample were precipitated with trichloroacetic acid to a final  
124 concentration of 10% in gentle shaking for 1 h and then centrifuged at 15,000 g for 30 min at 4 °C. The  
125 protein pellets were washed three times with cold absolute acetone, air-dried and dissolved in a  
126 rehydration buffer containing 7 M urea, 2 M thiourea, 4% 3-[(3-cholamidopropyl) dimethylammonio]-  
127 1-propanesulfonate (CHAPS), 65 mM dithiothreitol (DTT) and 0.8% resolytes (pH 3-10) before  
128 loading onto immobilized pH gradient (IPG) strips (non-linear pH gradient 3–10, 17 cm long)  
129 (BioRad). IPG strips were rehydrated and equilibrated following the protocol described by Campos et  
130 al. (2013).

131

132 The equilibrated IPG strips were placed on top of 10% acrylamide gel, and protein separation  
133 was run at 24 mA per gel for 6 h in Protean II XL (BioRad) in running buffer containing 25mM Tris,  
134 glycine 192 mM and SDS 0.1%, pH 8.8 (Campos et al., 2013). At the end of each run, the gels were  
135 stained by CBB. 2-DE gels were digitalized in a GS-800 calibrated densitometer (Bio-Rad) and the  
136 images analyzed by Progenesis SameSpot software (Non-Linear Dynamics) as described by Cruz De  
137 Carvalho et al., (2014).

138

### 139 *Protein identification by mass spectrometry*

140 Protein bands and spots were excised manually from the gels and subjected to in-gel tryptic  
141 digestion as previously described (Bellei et al., 2013). After digestion, the peptides were analyzed by a

142 Nano LC-CHIP-MS system (ESI-Q-TOF 6520, Agilent Technologies). Data were acquired in data-  
143 dependent MS/MS mode in which, for each cycle, the three most abundant multiply charged peptides  
144 ( $2^+$  to  $4^+$ ), above an absorbance threshold of 200 in the MS scan ( $m/z$  full scan acquisition range from  
145 100 to 1700), were selected for MS/MS ( $m/z$  tandem mass spectrum acquisition range from 50 to  
146 1700). Each peptide was selected twice and then dynamically excluded for 0.1 min. Raw mass  
147 spectrometry data were processed with MassHunter Qualitative Analysis B.05.00 software to obtain the  
148 Mascot generic files for database searching using the following parameters: deisotope, Absolute Height  
149  $\geq 10$ , Relative Height  $\geq 0.1\%$  of largest peak.

150

#### 151 *Statistical analysis*

152 Data were analyzed with statistical software (MedCalc Statistical Software version 12.7.5) and  
153 expressed as median and (range) or mean  $\pm$  standard deviation (SD). The different variables (UPC, age,  
154 number of bands) were compared using the Kruskal-Wallis one-way analysis of variance assuming  $P$   
155  $< 0.05$  as a significant probability.

156

#### 157 *Animal experimentation disclosure*

158 The study was approved by our Institutional Scientific Ethical Committee for Animal Testing  
159 (approval number 8-72-2012; date of approval 01 October 2012). AMA holds a FELASA grade C  
160 certificate enabling the design and conduction of animal experimentation under EU law.

161

## 162 **Results**

### 163 *Animal selection and UPC*

164 Out of the 44 entire domestic shorthair cats selected for the healthy group, 21 were excluded  
165 due to inadequate USG ( $< 1.035$ ), UPC  $> 0.2$ , a urinalysis abnormality (glucosuria, haematuria,



166 haemoglobinuria) or an active sediment (>5 white blood cells per high power field or bacteriuria). The  
167 remaining 23 cats (8 males, 15 females) were included in the study as the healthy group. The median  
168 age was 24 months (6-168) and median UPC was 0.11 (0.06-0.19).

169

170 Seventeen cats (5 neutered females, 8 neutered males and 4 entire males) were included in the  
171 CKD group. CKD cats were significantly older with a median age of 168 (60-240;  $P < 0.01$ ) and had a  
172 significantly increased UPC value (median 0.9; 0.25-6.5) than healthy cats ( $P < 0.01$ ). All urine samples  
173 had inactive sediment and were negative on urine culture. Serum biochemistry and urinalysis data are  
174 reported in Table 1.

175

#### 176 *ID-SDS-PAGE*

177 Representative gels and pherograms from healthy and CKD cats are shown in Fig. 1. We  
178 separated  $32 \pm 6$  protein bands in the urine of healthy cats. The majority had a molecular weight (MW)  
179 of between 10 and 80 kDa. The CKD group had a greater inter-individual variability and typical tubular  
180 pattern, characterized by low MW protein bands. A significant decrease in the total number of bands  
181 ( $25 \pm 6$ ) ( $P < 0.01$ ) was observed (Fig. 2a), particularly at MW higher than 100 kDa ( $P < 0.01$ ) (Figs. 1b  
182 and 2b).

183

184 No significant differences were found between pooled urine samples collected from healthy  
185 males and females. The most representative and reproducible protein bands from healthy and CKD  
186 samples ( $n = 16$ ) were excised from the gel for MS identification (Fig. 3).

187

#### 188 *2-DE and differential proteomics study*

189 Fig. 4 reports representative 2-D gels obtained from healthy (Fig. 4a) and CKD entire cats (Fig.  
190 4b). Serum biochemistry and urinalysis data are reported in Table 2. Of the 66 spots detected, 27  
191 showed differential expression ( $P<0.05$ ) between healthy and CKD samples; in particular, 18 spots  
192 were overrepresented in the CKD group and nine spots were increased in healthy animals. The  
193 remaining 39 spots were common and had similar expression levels. The nine most abundant common  
194 spots and the 27 differentially expressed spots were excised from the gels for MS identification.

195

#### 196 *Protein identification by mass spectrometry*

197 From the 16 bands excised from 1-D gels, 14 proteins were identified (Table 3). Of the 36 2-DE  
198 spots analysed, 20 yielded significant results by MS, allowing the successful identification of 13  
199 different proteins (Figs. 4a and b; Table 3). Albumin, cauxin, haemopexin and alpha-1 microglobulin  
200 precursor/bikunin (AMBP) were identified in spots characterized by different MW and/or isoelectric  
201 point. Seven proteins identified in 1-D gel were confirmed by 2-DE, namely uromodulin, albumin,  
202 transferrin, cauxin, haptoglobin, retinol binding protein (RBP) and immunoglobulin K light chain  
203 (IgK). Protein mass identification yielded a preliminary cat urine map, including 20 proteins that may  
204 be functionally classified as transport (25%), immune and cellular response (30%), protein metabolism  
205 (25%), and cellular communication and growth (15%) (Fig. 5a). Most of the identified proteins were  
206 classified as extracellular (75%) (Fig. 5b).

207

208 Cystatin M (CYSM), RBP, apolipoprotein-H (Apo-H), IgK and complement factor D (CFAD)  
209 were overrepresented in CKD samples, while alpha-2-macroglobulin (A2M), uromodulin, cauxin,  
210 inter-alpha-trypsin inhibitor heavy chain (ITIH4), pro-epidermal growth factor (EGF), angiotensin-  
211 converting enzyme (ACE2) and perlecan were underrepresented (Table 4). Examples of differentially

212 expressed spots are reported in Fig. 4c. The other proteins did not show significant differences between  
213 groups.

214

## 215 **Discussion**

216 The first aim of our research was to characterize the urine proteome in healthy cats and  
217 establish the proteome reference map. 1-D-SDS-PAGE yielded an overall view of the protein profile  
218 and resulted in a useful diagnostic tool that could help clinicians in qualitative evaluation of  
219 proteinuria. 2-DE was essential in fractionation of the complex urine proteome producing a reference  
220 map that included 20 proteins derived from either plasma ultrafiltration or kidney secretion, in  
221 accordance with data reported in humans (Adachi et al., 2006; Candiano et al., 2010; He et al., 2012)  
222 and dogs (Nabity et al., 2011; Brandt et al., 2014).

223

224 The most abundant protein was cauxin, a serine esterase produced by healthy tubular cells,  
225 specifically excreted in urine of cats and probably involved in the synthesis of feline pheromone  
226 (Miyazaki et al., 2006). Most of the other proteins identified were involved in protein metabolism,  
227 immune response and transport. Regarding protein metabolism, we found several protease inhibitors  
228 (A2M, A1AT, ITIH4) that may play an important role in protecting the kidney from proteolytic  
229 damage. Among the proteins involved in immune and cellular defence response, we identified protein  
230 AMPB, IgK and uromodulin. In contrast to dogs (Nabity et al., 2011; Brandt et al., 2014; Miller et al.,  
231 2014) and humans (Lhotta, 2010), uromodulin is not the most abundant urine-specific protein in cats.

232

233 The transport proteins, albumin, transferrin, haemopexin and haptoglobin all derive from  
234 plasma and have been identified as common components of urine also from healthy humans (Candiano  
235 et al., 2010). The presence of high MW plasma proteins, such as transferrin and A2M, in cat urine

236 could contradict the paradigm of glomerular selectivity that should be re-evaluated according to the  
237 findings of Candiano et al. (2010) and Brandt et al. (2014). However, a possible blood contamination of  
238 urine due to cystocentesis cannot be excluded. The remaining proteins, EGF, perlecan and fetuin-A, are  
239 involved in cell communication and growth. In particular, perlecan, a negatively charged proteoglycan  
240 of the glomerular filtration barrier, has also been identified in dog urine (Nabity et al., 2011).

241

242         Regarding the effect of CKD on the urine cat proteome, we identified 13 proteins differentially  
243 represented that could be studied as putative biomarkers of nephropathy (Table 4). Our inclusion  
244 criteria led to the selection of proteinuric late stage CKD patients and based on UPC values a severe  
245 glomerular involvement could be hypothesised. However, most of these differentially expressed  
246 proteins are indicative of tubular dysfunction (e.g. RBP, CYSM, uromodulin and cauxin).

247

248         Among the overrepresented proteins, RBP is a low MW protein belonging to the family of  
249 lipocalins and is involved in plasma retinol transport. An increase in RBP is considered a biomarker of  
250 tubulointerstitial damage in humans and a significant correlation between urinary RBP and kidney  
251 interstitial fibrosis was recently demonstrated in CKD patients (Pallet et al., 2014). Elevated RBP in  
252 case of tubular damage has also been reported in dogs (Smets et al., 2010; Nabity et al., 2011). On the  
253 basis of our results, RBP can be considered an appealing marker to diagnose and monitor CKD in cats,  
254 as previously suggested by van Hoek et al. (2008).

255

256         CYSM belongs to the cystatin family, a class of lysosomal cysteine protease inhibitors, and is  
257 considered a major regulator of epidermal cornification and desquamation (Brocklehurst and Philpott,  
258 2013). To our knowledge, CYSM has never been found in urine, while an increase in the more widely

259 studied cystatin C has been correlated with tubular dysfunction in humans, dogs (Monti et al., 2012)  
260 and cats (Ghys et al., 2014); further studies are needed to clarify the role of CYSM in urine.

261

262 Apo-H (beta-2-glycoprotein 1) is a single chain multifunctional apolipoprotein also expressed  
263 in kidney tubular epithelium and involved in clotting mechanisms and lipid metabolism (Klaerke et al.,  
264 1997). The increase in urinary Apo-H in diabetic patients has been proposed as a marker of tubular  
265 dysfunction (Lapsley et al., 1993), and recent studies focused on the increased levels of IgA anti-Apo-  
266 H in CKD patients (Serrano et al., 2014); the role of this protein in cat urine is still unknown. The last  
267 two overrepresented proteins in CKD cats, namely CFAD and IgK, are involved in the immune  
268 response. CFAD is a serine protease synthesized mainly by adipocytes and macrophages belonging to  
269 the alternative complement pathway. The only report of this protein in urine regards a significant  
270 increase in human patients with preeclampsia (Wang et al., 2014).

271

272 Among the underrepresented proteins, there were significant decreases in uromodulin, cauxin  
273 and perlecan. Uromodulin is a 95-kDa glycoprotein exclusively synthesized by the cells of the thick  
274 ascending limb. Its exact molecular function is still unknown, but it is thought to be a potent immuno-  
275 regulatory protein: recent studies have hypothesized that uromodulin entering the renal interstitium  
276 through the damaged tubuli can stimulate the cells of the immune system causing inflammation and  
277 CKD progression (El-Achkar and Wu, 2012). The decrease in uromodulin was previously observed  
278 also in dogs affected by leishmaniasis (Buono et al., 2012), suggesting its use as a biomarker of renal  
279 damage in small animals.

280

281 2-DE was essential in obtaining the separation of albumin from cauxin, demonstrating a  
282 significant decrease of cauxin; however a possible influence of the entire/neutered status cannot be

283 completely excluded. Though Jepson et al. (2010) showed a weak correlation between cauxin and the  
284 onset of azotemia, our data, according to Miyazaki et al. (2007), suggest that this protein could be a  
285 promising biomarker for the determination of tubular damage in CKD cats, particularly in entire males.

286

287 The decrease of perlecan in human urine is associated with damage in the glomerular  
288 compartment (Ebefors et al., 2011) and could also suggest glomerular involvement in cats affected by  
289 renal disease. The remaining underrepresented proteins are involved in protein metabolism or cellular  
290 defence and communication. In particular, the decrease in the protease inhibitors A2M and ITIH4 could  
291 indicate a role in the pathophysiology of CKD. In support of this mechanism, intensive protein  
292 degradation has also been reported to occur in the urine of humans with CKD (Mullen et al., 2011).  
293 This finding is in accordance with the increased protein fragmentation, especially of albumin, found in  
294 our study.

295

296 Although the proteomic approach we applied led to a preliminary feline urine proteome map  
297 and to the identification of new putative biomarkers of nephropathy, our study presented some  
298 limitations. To obtain samples with an adequate amount of proteins, we selected proteinuric cats with  
299 advanced stages of CKD. Although we excluded patients with possible primary glomerular  
300 involvement, we cannot state that all cats included in the study had the same underlying renal  
301 pathophysiological condition. Therefore, further studies are needed to confirm our results and to  
302 evaluate urine proteome also in non-proteinuric CKD cats. Moreover, the differences in age and neuter  
303 status between healthy and CKD cats could be considered minor limitations. In our study the age-  
304 related changes should have been minimized by the selection of proteinuric cats with advanced stages  
305 of CKD and the neuter/entire influence reduced by the exclusion of borderline and proteinuric healthy  
306 male entire cats.

307

**308 Conclusions**

309 Our work has produced a reference map of the normal urine proteome in cats and can be  
310 considered the starting point for future studies. Moreover, this is the first research linking of 13  
311 differentially represented urine proteins with CKD in cats. The different amounts of uromodulin,  
312 cauxin, CFAD, Apo-H, RBP and CYSM confirm tubulointerstitial damage in CKD cats and suggest  
313 that these proteins are candidate biomarkers to be investigated further. Our data on the proteins most  
314 represented in the cat urine proteome and their changes in CKD could be useful in the advancement of  
315 research focused on the discovery of new biomarkers for later use in clinical practice. In particular,  
316 uromodulin, cauxin and perlecan, which are specifically secreted in urine, could help in the evaluation  
317 of feline renal function.

318

319

320

321

**322 Acknowledgements**

323 This study was supported by a grant from the University of Bologna (RFO) to GI. AMA is  
324 supported by grant SFRH/BPD/90916/2012 from Fundação para a Ciência e a Tecnologia (Lisbon,  
325 Portugal), AC by the contract work Ciência 2007 from FCT. AMA, AC, EF and GI are members of  
326 COST Action FA1002 – Proteomics in Farm Animals<sup>3</sup> funded by the European Science Foundation  
327 and to whom networking support is acknowledged.

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<sup>3</sup> See: [www.cost-FAProteomics.org](http://www.cost-FAProteomics.org)

330 **References**

- 331 Adachi, J., Kumar, C., Zhang, Y., Olsen, V.J., Mann, M., 2006. The human urinary proteome contains  
 332 more than 1500 proteins, including a large proportion of membrane proteins. *Genome Biology* 7,  
 333 R80.  
 334
- 335 Almeida, A.M., Bassols, A., Bendixen, E., Bhide, M., Ceciliani, F., Cristobal, S., Hollung, K., Lisacek,  
 336 F., Mazzucchelli, G., McLaughlin M. et al., 2015. Invited review: advances in proteomics for  
 337 animal and food sciences. *Animal* 9, 1-17.  
 338
- 339 Bartges, J.W., 2012. Chronic kidney disease in dogs and cats. *Veterinary Clinics of North America:*  
 340 *Small Animal Practice* 42, 669-692.  
 341
- 342 Bellei, E., Monari, E., Cuoghi, A., Bergamini, S., Guerzoni, S., Ciccarese, M., Ozben, T., Tomasi, A.,  
 343 Pini, L.A., 2013. Discovery by a proteomic approach of possible early biomarkers of drug-induced  
 344 nephrotoxicity in medication-overuse headache. *The Journal of Headache and Pain* 14, 6.  
 345
- 346 Bertoldi, C., Bellei, E., Pellacani, C., Ferrari, D., Lucchi, A., Cuoghi, A., Bergamini, S., Cortellini, P.,  
 347 Tomasi, A., Zaffe, D., et al., 2013. Non-bacterial protein expression in periodontal pockets by  
 348 proteome analysis. *Journal of Clinical Periodontology*. 40, 573-582.  
 349
- 350 Brandt, L.E., Ehrhart, E.J., Scherman, H., Olver, C.S., Bohn, A.A., Prenni, J.E., 2014. Characterization  
 351 of the canine urinary proteome. *Veterinary Clinical Pathology* 43, 193-205.  
 352
- 353 Brocklehurst, K., Philpott, M.P., 2013. Cysteine proteases: mode of action and role in epidermal  
 354 differentiation. *Cell and Tissue Research* 351, 237-244.  
 355
- 356 Buono, A., Duque, J., Ferlizza, E., Zaragoza, C., Barrera, R., Isani, G., Dondi, F., 2012. Non-invasive  
 357 diagnostic evaluation including qualitative proteinuria to detect an early renal damage in canine  
 358 leishmaniasis. In: *Proceedings of the European College of Veterinary Internal Medicine -*  
 359 *Companion Animal Congress, Maastricht, NL. Journal of Veterinary Internal Medicine* 26, 1537-  
 360 1538.  
 361
- 362 Campos, A., Puerto, M., Prieto, A., Cameán, A., Almeida, A.M., Coelho, A.V., Vasconcelos, V., 2013.  
 363 Protein extraction and two-dimensional gel electrophoresis of proteins in the marine mussel  
 364 *Mytilus galloprovincialis*: an important tool for protein expression studies, food quality and safety  
 365 assessment. *Journal of the Science of Food and Agriculture* 93, 1779-1787.  
 366
- 367 Candiano, G., Santucci, L., Petretto, A., Bruschi, M., Dimuccio, V., Urbani, A., Bagnasco, S.,  
 368 Ghiggeri, G.M., 2010. 2D-electrophoresis and the urine proteome map: where do we stand?  
 369 *Journal of Proteomics* 73, 829-844.  
 370
- 371 Cruz De Carvalho, R., Bernardes Da Silva, A., Soares, R., Almeida, A.M., Coelho, A.V., Marques Da  
 372 Silva, J., Branquinho, C., 2014. Differential proteomics of dehydration and rehydration in  
 373 bryophytes: evidence towards a common desiccation tolerance mechanism. *Plant, Cell and*  
 374 *Environment* 37, 1499-1515.  
 375



- 376 Ebefors, K., Granqvist, A., Ingelsten, M., Mölne, J., Haraldsson, B., Nyström, J., 2011. Role of  
377 glomerular proteoglycans in IgA nephropathy. *PLoS ONE* 6, e18575.  
378
- 379 El-Achkar, T.M., Wu, X.R., 2012. Uromodulin in kidney injury: an instigator, bystander, or protector?  
380 *American Journal of Kidney Disease* 59, 452-61.  
381
- 382 Ghys, L.F.E., Meyer, E., Paepe, D., Delanghe, J., Daminet, S., 2014. Analytical validation of a human  
383 particle-enhanced nephelometric assay for cystatin C measurement in feline serum and urine.  
384 *Veterinary Clinical Pathology* 43, 226-234.  
385
- 386 He, W., Huang, C., Luo, G., Dal Prà, I., Feng, J., Chen, W., Ma, L., Wang, Y., Chen, X., Tan, J. et al.,  
387 2012. A stable panel comprising 18 urinary proteins in the human healthy population. *Proteomics*  
388 12, 1059-1072.  
389
- 390 Jepson, R.E., Syme, H.M., Markwell, P., Miyazaki, M., Yamashita, T., Elliott, J., 2010. Measurement  
391 of urinary cauxin in geriatric cats with variable plasma creatinine concentrations and proteinuria  
392 and evaluation of urine cauxin-to-creatinine concentration ratio as a predictor of developing  
393 azotemia. *American Journal of Veterinary Research* 71, 982-987.  
394
- 395 Jepson, R., Coulton, G., Cowan, M., Markwell, P., Syme, H.M., Elliott, J., 2013. Evaluation of mass  
396 spectrometry of urinary proteins and peptides as biomarkers for cats at risk of developing  
397 azotemia. *American Journal of Veterinary Research* 74, 333-342.  
398
- 399 Klaerke, D.A., Røjkjaer, R., Christensen, L., Schousboe, I., 1997. Identification of  $\beta$ 2-glycoprotein I as  
400 a membrane-associated protein in kidney: purification by calmodulin affinity chromatography.  
401 *Biochimica et Biophysica Acta* 1339, 203-216.  
402
- 403 Lapsley, M., Flynn, F. V., Sansom, P.A., 1993. Beta 2-Glycoprotein-1 (apolipoprotein H) excretion and  
404 renal tubular malfunction in diabetic patients without clinical proteinuria. *Journal of Clinical*  
405 *Pathology* 46, 465-469.  
406
- 407 Lemberger, S.I.K., Deeg, C.A., Hauck, S.M., Amann, B., Hirmer, S., Hartmann, K., Dorsch, R., 2011.  
408 Comparison of urine protein profiles in cats without urinary tract disease and cats with idiopathic  
409 cystitis, bacterial urinary tract infection, or urolithiasis. *American Journal of Veterinary Research*  
410 72, 1407-1415.  
411
- 412 Lhotta, K., 2010. Uromodulin and chronic kidney disease. *Kidney and Blood Pressure Research* 33,  
413 393-398.  
414
- 415 Miller, I., Preßlmayer-Hartler, A., Wait, R., Hummel, K., Sensi, C., Eberini, I., Razzazi-Fazeli, E.,  
416 Gianazza, E., 2014. In between - Proteomics of dog biological fluids. *Journal of Proteomics* 106,  
417 30-45.  
418
- 419 Mischke, R., 2011. Assessing proteinuria in the cat: an innovative tip. *The Veterinary Journal* 189, 243-  
420 244.  
421

- 422 Miyazaki, M., Yamashita, T., Hosokawa, M., Taira, H., Suzuki, A., 2006. Species-, sex-, and age-  
423 dependent urinary excretion of cauxin, a mammalian carboxylesterase. *Comparative Biochemistry  
424 and Physiology Part B: Biochemistry and Molecular Biology* 145, 270-277.  
425
- 426 Miyazaki, M., Soeta, S., Yamagishi, N., Taira, H., Suzuki, A., Yamashita, T., 2007. Tubulointerstitial  
427 nephritis causes decreased renal expression and urinary excretion of cauxin, a major urinary  
428 protein of the domestic cat. *Research in Veterinary Science* 82, 76-79.  
429
- 430 Miyazaki, M., Fujiwara, K., Suzuta, Y., Wakamatsu, N., Taira, H., Suzuki, A., Yamashita, T., 2011.  
431 Screening for proteinuria in cats using a conventional dipstick test after removal of cauxin from  
432 urine with a *Lens culinaris* agglutinin lectin tip. *The Veterinary Journal* 189, 312-317.  
433
- 434 Monti, P., Benckekroun, G., Berlato, D., Archer, J., 2012. Initial evaluation of canine urinary cystatin C  
435 as a marker of renal tubular function. *Journal of Small Animal Practice* 53, 254-259.  
436
- 437 Mullen, W., Delles, C., Mischak, H., 2011. Urinary proteomics in the assessment of chronic kidney  
438 disease. *Current Opinion in Nephrology and Hypertension* 20, 654-661.  
439
- 440 Nability, M.B., Lees, G.E., Dangott, L.J., Cianciolo, R., Suchodolski, J.S., Steiner, J.M., 2011.  
441 Proteomic analysis of urine from male dogs during early stages of tubulointerstitial injury in a  
442 canine model of progressive glomerular disease. *Veterinary Clinical Pathology* 40, 222-236.  
443
- 444 Pallet, N., Chauvet, S., Chassé, J.-F., Vincent, M., Avillach, P., Levi, C., Meas-Yedid, V., Olivo-Marin,  
445 J.C., Nga-Matsogo, D., Beaune, P. et al., 2014. Urinary retinol binding protein is a marker of the  
446 extent of interstitial kidney fibrosis. *PLoS ONE* 9, e84708.  
447
- 448 Polzin, D.J., 2011. Chronic kidney disease in small animals. *Veterinary Clinics of North America:  
449 Small Animal Practice* 41, 15-30.  
450
- 451 Schaefer, H., Kohn, B., Schweigert, F.J., Raila, J., 2011. Quantitative and qualitative urine protein  
452 excretion in dogs with severe inflammatory response syndrome. *Journal of Veterinary Internal  
453 Medicine* 25, 1292-1297.  
454
- 455 Serrano, M., Martínez-Flores, J.A., Castro, M.J., García, F., Lora, D., Pérez, D., Gonzalez, E., Paz-  
456 Artal, E., Morales, J.M., Serrano, A., 2014. Renal transplantation dramatically reduces IgA anti-  
457 beta-2-glycoprotein I antibodies in patients with endstage renal disease. *Journal of Immunology  
458 Research* 2014, 641962.  
459
- 460 Smets, P.M.Y., Meyer, E., Maddens, B.E.J., Duchateau, L., Daminet, S., 2010. Urinary markers in  
461 healthy young and aged dogs and dogs with chronic kidney disease. *Journal of Veterinary Internal  
462 Medicine* 24, 65-72.  
463
- 464 Tesch, G.H., 2010. Review: serum and urine biomarkers of kidney disease: a pathophysiological  
465 perspective. *Nephrology* 15, 609-616.  
466
- 467 Van Hoek, I., Daminet, S., Notebaert, S., Janssens, I., Meyer, E., 2008. Immunoassay of urinary retinol  
468 binding protein as a putative renal marker in cats. *Journal of Immunological Methods* 329, 208-  
469 213.

470  
471 Wang, T., Zhou, R., Gao, L., Wang, Y., Song, C., Gong, Y., Jia, J., Xiong, W., Dai, L., Zhang, L., et  
472 al., 2014. Elevation of urinary adipsin in preeclampsia: correlation with urine protein  
473 concentration and the potential use for a rapid diagnostic test. *Hypertension* 64, 846-851.  
474

475 **Table 1**

476 Clinical data for cats affected by CKD (*n*=17).

477

Signalment	Mean ± SD	<i>n</i>	
Age in months	160±64		
Female (entire/neutered)		5 (0/5)	
Male (entire/neutered)		12 (4/8)	
Serum biochemistry	Mean ± SD	<i>n</i> (%) < or >RI	RI
Total Proteins (g/dL)	7.9±0.8	6(35)>	6.0-8.0
Albumin (g/dL)	3±0.4	4(24)>	2.1-3.3
Creatinine (mg/dL)	5.9±3.6	17(100)>	0.8-1.6
Urea (mg/dL)	264±148	16(94)>	15-60
Phosphorus (mg/dL)	9.5±5.7	9(54)>	2.9-8.3
Urine biochemistry	Mean ± SD	<i>n</i> (%) < or >RI	RI
UPC	1.29±1.52	14(82)>	<0.4
USG	1.018±0.012	15(88)<	>1.035 <sup>1</sup>
IRIS Stage		<i>n</i> (%)	
II		4(24)	
III		4(24)	
IV		9(53)	
Clinical signs		<i>n</i> (%)	
Inappetence/anorexia		15(88)	
Polyuria/polydipsia		11(65)	
Depression		7(41)	
Weight loss		4(24)	
Abnormal renal palpation		3(18)	
Oral lesions		3(18)	
Vomiting		2(12)	
Weakness		2(12)	
Dehydration		2(12)	
Diarrhoea		1(6)	
Blindness		1(6)	

478 RI, reference interval; UPC, urine protein to creatinine ratio; USG, urine specific gravity.

479 <sup>1</sup> Considered as adequate USG in cats

480

481

482 **Table 2**

483 Clinical data for healthy and CKD cats selected for 2-DE.

	Gender	Age (months)	TP (g/dL)	ALB (g/dL)	Creatinine (mg/dL)	Urea (mg/dL)	P (mg/dL)	UPC	USG	IRIS stage
RI			6.0-8.0	2.1-3.3	0.8-1.6	15-60	2.9-8.3	<0.4	>1.035 <sup>1</sup>	
CKD										
1	M	96	6.35	2.35	1.76	97	4.9	0.50	1.020	II
2	C	216	8	3	4.3	195	5.5	1.50	1.018	III
3	C	160	8.8	2.65	5.23	401	18.3	6.30	1.022	IV
4	M	170	9	2.8	8.9	474	17	3.50	1.014	IV
Healthy										
1	M	6	6.76	2.4	0.95	56	4.3	0.19	1048	
2	M	24	7.12	3	1.35	43	3.2	0.13	1056	
3	M	12	6.5	2.8	1.5	25	6.8	0.08	1072	
4	M	6	7.6	2.9	1.24	50	5.4	0.14	1044	

484

485 TP, serum total protein; ALB, serum albumin; P, serum phosphorus; UPC, urine protein to creatinine ratio; USG, urine  
 486 specific gravity; RI, reference interval; M, entire male; C, neutered male.

487

488

489 <sup>1</sup> Considered as adequate USG in cats

490

491 **Table 3**

492 Proteins identified in cat urine by mass spectrometry.

Band <sup>a</sup> 1-DE	Entry name <sup>b</sup>	Protein full name	MW <sup>c</sup> (kDa)	pI	Score <sup>d</sup>	Pept. <sup>e</sup>	Seq. <sup>f</sup>	Sign. Seq. <sup>g</sup>	Identity <sup>h</sup>
1	TRFE_BOVIN	Serotransferrin	79.9	6.75	88	15	7	3	73
2	EST5A_FELCA	Carboxylesterase 5A	60.9	5.58	238	27	10	6	100
	ALBU_FELCA	Serum albumin	70.6	5.46	135	21	8	6	100
3	ALBU_FELCA	Serum albumin	70.6	5.46	346	37	16	10	100
	EST5A_FELCA	Carboxylesterase 5A	60.9	5.58	41	8	4	2	100
4	KV1_CANFA	Ig kappa chain V region GOM	12.1	6.41	91	3	2	1	71
	IGLL5_HUMAN	Immunoglobulin lambda-like polypeptide 5	23.4	9.08	66	11	1	1	79
5	ALBU_FELCA	Serum albumin	70.6	5.46	59	9	6	2	100
	ALBU_FELCA	Serum albumin	70.6	5.46	1340	115	34	25	100
6	RET4_HORSE	Retinol-binding protein 4	23.3	5.28	1121	42	6	4	93
7	CYTM_HUMAN	Cystatin-M	16.5	7.0	71	3	2	1	79
8	A2MG_BOVIN	Alpha-2-macroglobulin	168.9	5.71	121	9	4	1	75
	ALBU_FELCA	Serum albumin	70.6	5.46	115	18	9	4	100
9	ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	103.5	6.51	70	9	2	2	73
	ACE2_FELCA	Angiotensin-converting enzyme 2	93.1	5.64	178	15	6	5	100
10	UROM_CANFA	Uromodulin	72.9	4.94	112	20	4	4	86
	EGF_FELCA	Pro-epidermal growth factor	137.3	5.8	83	13	7	4	100
11	ALBU_FELCA	Serum albumin	70.6	5.46	147	24	11	7	100
	EST5A_FELCA	Carboxylesterase 5A	60.9	5.58	145	20	8	2	100
12	HPT_CANFA	Haptoglobin	36.9	5.72	80	27	8	6	90
	EST5A_FELCA	Carboxylesterase 5A	60.9	5.58	102	16	7	3	100
13	IGLL5_HUMAN	Immunoglobulin lambda-like polypeptide 5	23.4	9.08	115	16	1	1	100
	EST5A_FELCA	Carboxylesterase 5A	60.9	5.58	254	30	12	6	100
14	TRFE_PIG	Serotransferrin	78.9	6.93	71	19	7	4	74
	ALBU_FELCA	Serum albumin	70.6	5.46	532	53	22	17	100
15	EST5A_FELCA	Carboxylesterase 5A	60.9	5.58	439	68	16	9	100
	ALBU_FELCA	Serum albumin	70.6	5.46	5932	346	51	42	100
16	EST5A_FELCA	Carboxylesterase 5A	60.9	5.58	1941	157	24	23	100
	A1AT_CHLAE	Alpha-1-antitrypsin	44.6	5.75	109	11	3	2	71
<b>Spot<sup>a</sup> 2-DE</b>									
1	UROM_CANFA	Uromodulin	72.9	4.94	130	36	6	3	86
2	ALBU_FELCA	Serum albumin	70.6	5.46	2383	196	39	28	100
3	ALBU_FELCA	Serum albumin	70.6	5.46	2133	208	35	29	100
4	EST5A_FELCA	Carboxylesterase 5A	60.9	5.58	524	66	14	10	100
5	EST5A_FELCA	Carboxylesterase 5A	60.9	5.58	447	89	14	10	100

6	TRFE_PIG	Serotransferrin	78.9	6.93	114	31	9	5	74
7	FETUA_HUMAN	Fetuin-A	40.1	5.43	141	34	6	4	70
8	APOH_CANFA	Apolipoprotein H	39.7	8.51	162	21	4	4	88
9	HPT_BOVIN	Haptoglobin	45.6	7.83	72	6	2	2	78
10	AMBP_BOVIN	Protein AMBP	40.1	7.81	141	5	1	1	78
11	AMBP_BOVIN	Protein AMBP	40.1	7.81	150	6	1	1	78
12	AMBP_BOVIN	Protein AMBP	40.1	7.81	274	11	1	1	78
13	PGBM_HUMAN	Perlecan	479.3	6.06	134	19	3	2	91
14	HEMO_PONAB	Hemopexin	52.3	6.44	73	25	3	1	83
15	HEMO_PONAB	Hemopexin	52.3	6.44	97	25	3	1	83
16	ALBU_FELCA	Serum albumin	70.6	5.46	1585	187	40	25	100
17	APOH_CANFA	Apolipoprotein H	39.7	8.51	119	16	5	4	88
18	ALBU_FELCA	Serum albumin	70.6	5.46	69	10	7	3	100
19	KV1_CANFA	Ig kappa chain V region GOM	12.1	6.41	111	4	2	2	71
	CFAD_PIG	Complement factor D	28.3	6.59	54	9	2	2	86
20	RET4_HUMAN	Retinol-binding protein 4	23.3	5.76	167	27	8	3	94

493

494

<sup>a</sup> Number of the identified band or spot as marked in Figs. 3 and 4, respectively.

495

<sup>b</sup> Protein entry name from UniProt knowledge database.

496

<sup>c</sup> Theoretical protein molecular weight.

497

<sup>d</sup> The highest scores obtained with Mascot search engine.

498

<sup>e</sup> Peptides: total number of peptides matching the identified proteins.

499

<sup>f</sup> Sequence: total number of sequences matching the identified proteins.

500

<sup>g</sup> Significant Sequences: total number of significant sequences matching the identified proteins.

501

<sup>h</sup> Percentage of identical amino acids between the identified protein and the respective cat protein.

502

503 **Table 4**

504 Differentially expressed proteins identified by mass spectrometry (ESI-Q-TOF).

Band <sup>a</sup> 1-DE	Entry name <sup>b</sup>	Protein full name	CKD vs. healthy <sup>c</sup>	Molecular function <sup>d</sup>	Biological process <sup>e</sup>
6	RET4_HUMAN	Retinol-binding protein 4	Up	Transporter	Transport
7	CYTM_HUMAN	Cystatin-M	Up	Protease inhibitor	Protein metabolism
8	A2MG_BOVIN	Alpha-2-macroglobulin	Down	Protease inhibitor	Protein metabolism
9	ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	Down	Protease inhibitor	Protein metabolism
10	ACE2_FELCA	Angiotensin-converting enzyme 2	Down	Protease-carboxypeptidase activity	Protein metabolism
	UROM_CANFA	Uromodulin	Down	Unknown	Cellular defence response
	EGF_FELCA	Pro-epidermal growth factor	Down	Growth factor activity	Cell communication; Signal transduction
Spot <sup>a</sup> 2-DE					
1	UROM_CANFA	Uromodulin	Down	Unknown	Cellular defence response
2	ALBU_FELCA	Albumin	Down	Transporter	Transport
4; 5	EST5A_FELCA	Carboxylesterase 5A	Down	Protease-hydrolase activity	Unknown
8; 17	APOH_CANFA	Apolipoprotein H	Up	Transporter	Transport
13	PGBM_HUMAN	Perlecan	Down	Extracellular matrix structural constituent	cell Growth/maintenance
16; 18	ALBU_FELCA	Albumin	Up	Transporter	Transport
19	KV1_CANFA	Ig kappa chain V region GOM	Up	Antigen binding	Immune response
	CFAD_PIG	Complement factor D	Up	Serine-type peptidase	Immune response
20	RET4_HUMAN	Retinol-binding protein 4	Up	Transporter	Transport

505

506 <sup>a</sup> Number of the identified band or spot as marked in Figs. 3 and 4 respectively.507 <sup>b</sup> Protein entry name from UniProt knowledge database.508 <sup>c</sup> Significantly ( $P < 0.05$ ) overrepresented (up) and underrepresented (down) proteins in CKD group respect to healthy.509 <sup>d</sup> Molecular function according to Gene Ontology and Human Reference Proteome Database.510 <sup>e</sup> Biological process according to Gene Ontology and Human Reference Proteome Database.

511



512 **Figure legends**

513

514 Fig. 1. 1D-SDS-PAGE of cat urine proteins. Two micrograms of proteins were loaded and stained with  
515 silver nitrate. Representative gel (lane 1, molecular weight marker; lanes 2-7, urine samples from CKD  
516 cats; lanes 8-9, healthy urine samples) (A) and pherograms (B) are shown.

517 Fig. 2. Comparison of the number of protein bands between healthy and CKD cats. (A) Total number  
518 of bands. (B) Number of bands with MW>100 kDa. Different lower cases indicate significant  
519 differences ( $P<0.01$ ).

520 Fig. 3. 1D-SDS-PAGE of urine samples from healthy and CKD cats, stained with Coomassie Blue.  
521 Lane 1, molecular weight marker; lanes 2-3, CKD urine samples; lanes 4-5, pools of urine from  
522 healthy females and males respectively. Rectangles and numbers indicate the bands that have been cut  
523 and identified by ESI-Q-TOF (Table 3).

524 Fig. 4. 2-DE of the urine proteome in healthy (A) and CKD (B) entire cats. White circles: spots with  
525 significantly greater intensity in healthy than in CKD; black circles: spots with significantly greater  
526 intensity in CKD; white rectangles: common spots without significant differences. (C) Examples of  
527 important differentially expressed proteins.

528 Fig. 5. Classification of the proteins identified according to Gene Ontology and the Human Reference  
529 Proteome Database (HRPD).

530

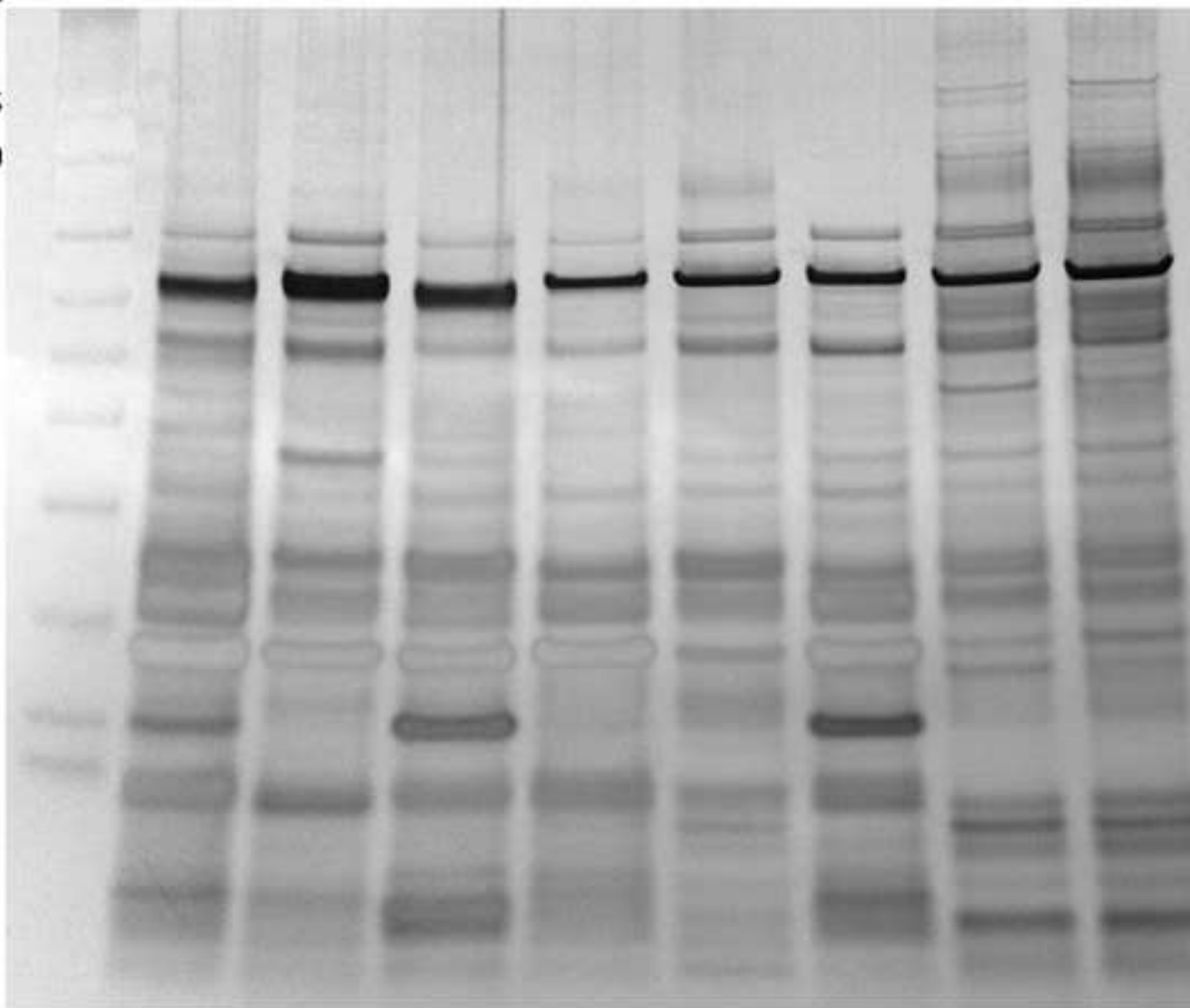
531

Figure1A

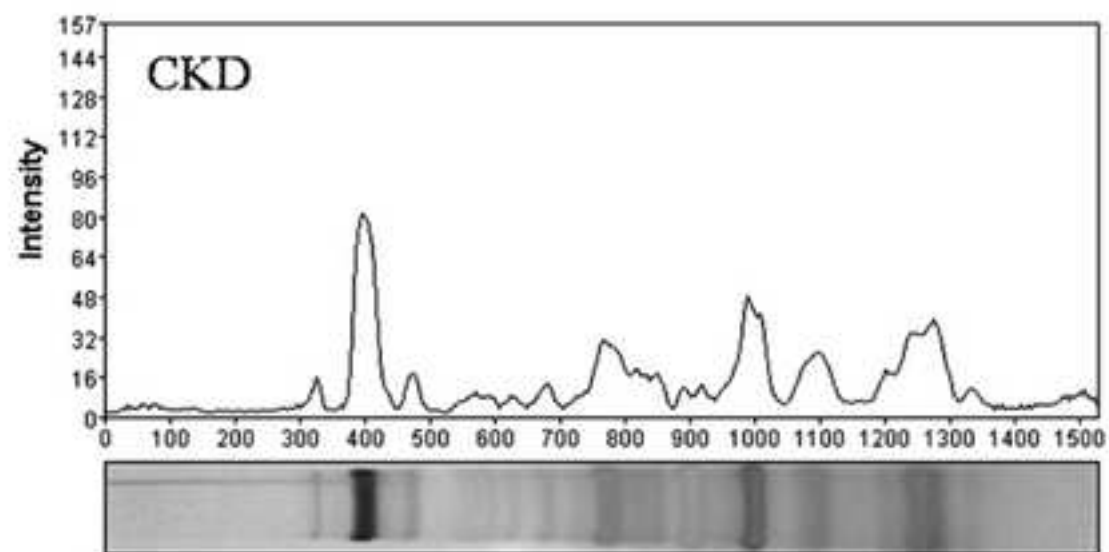
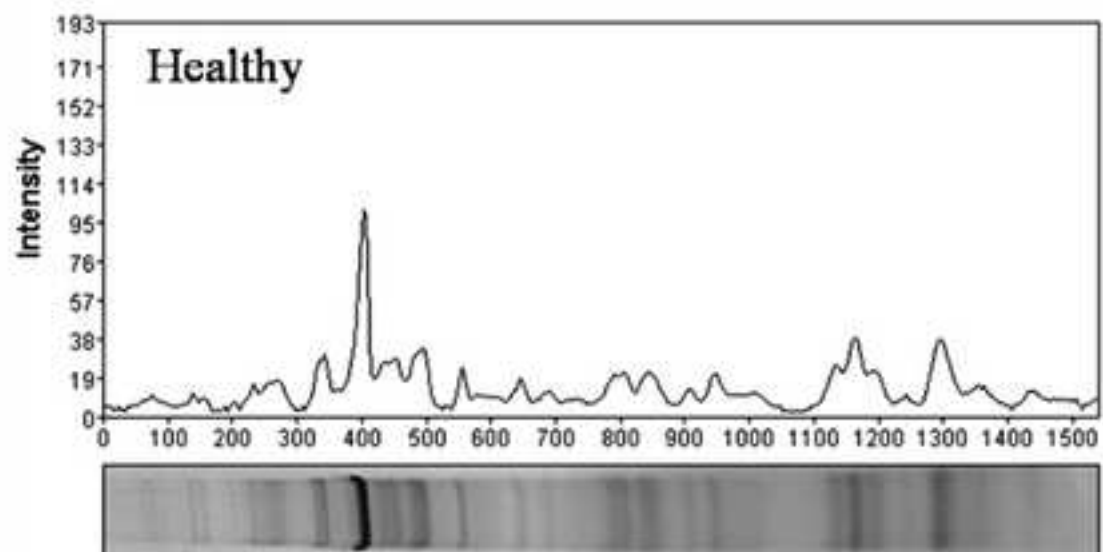
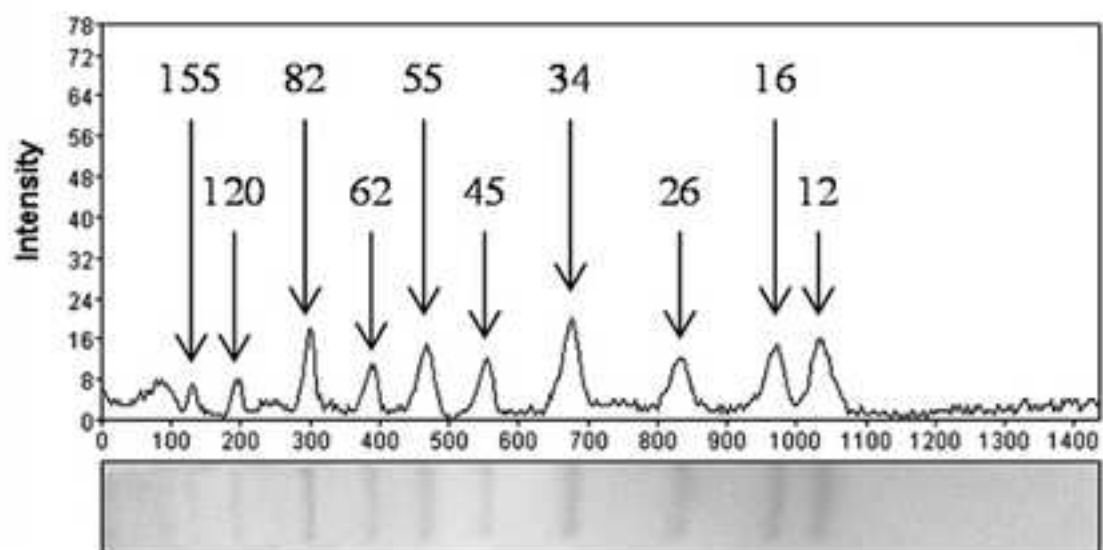
**A**

kDa

155  
120  
82  
62  
55  
45  
34  
26  
16  
12

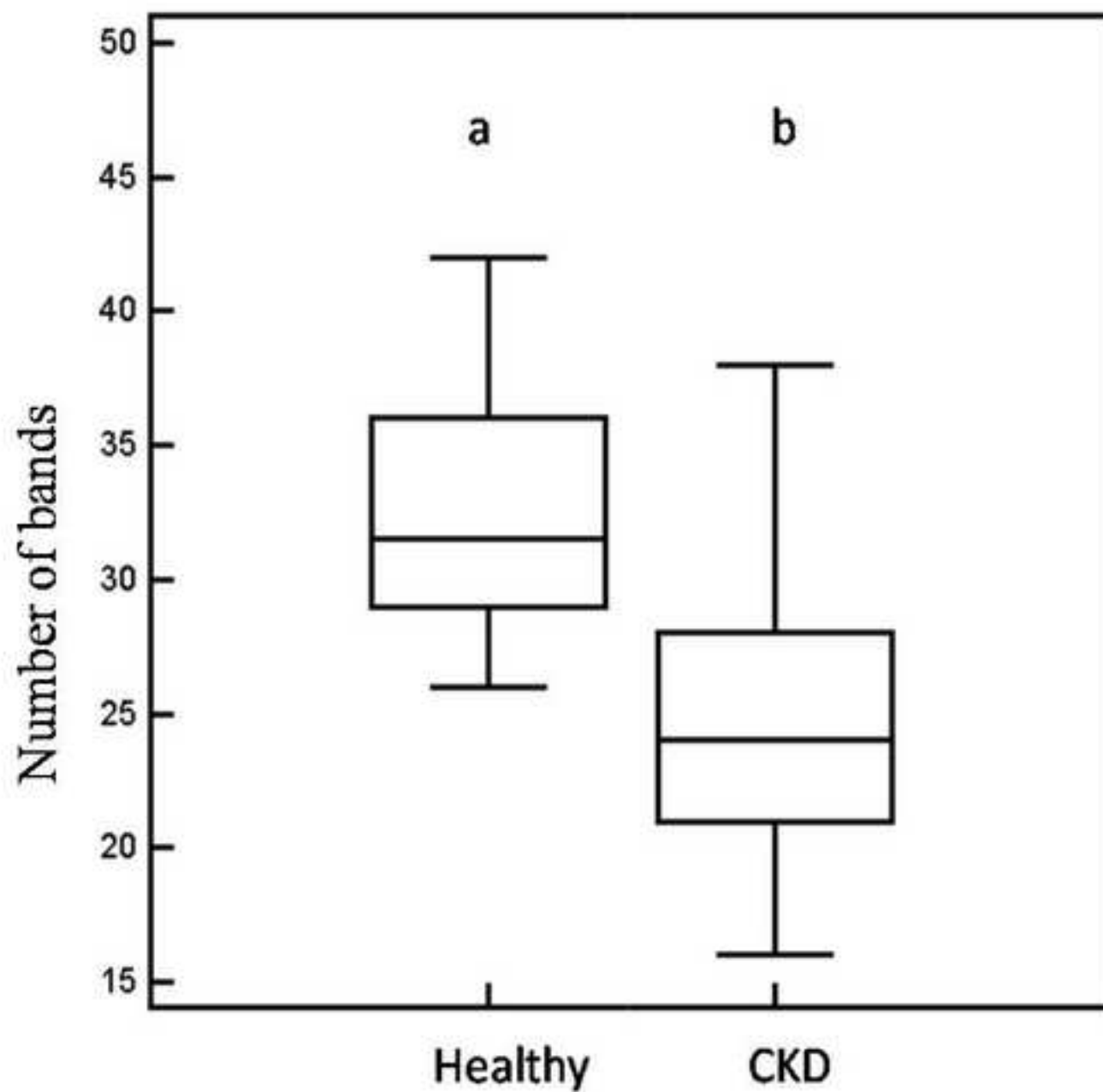


L1 L2 L3 L4 L5 L6 L7 L8 L9

**B**

A

Total number of bands



**B**

Number of bands MW&gt;100kDa

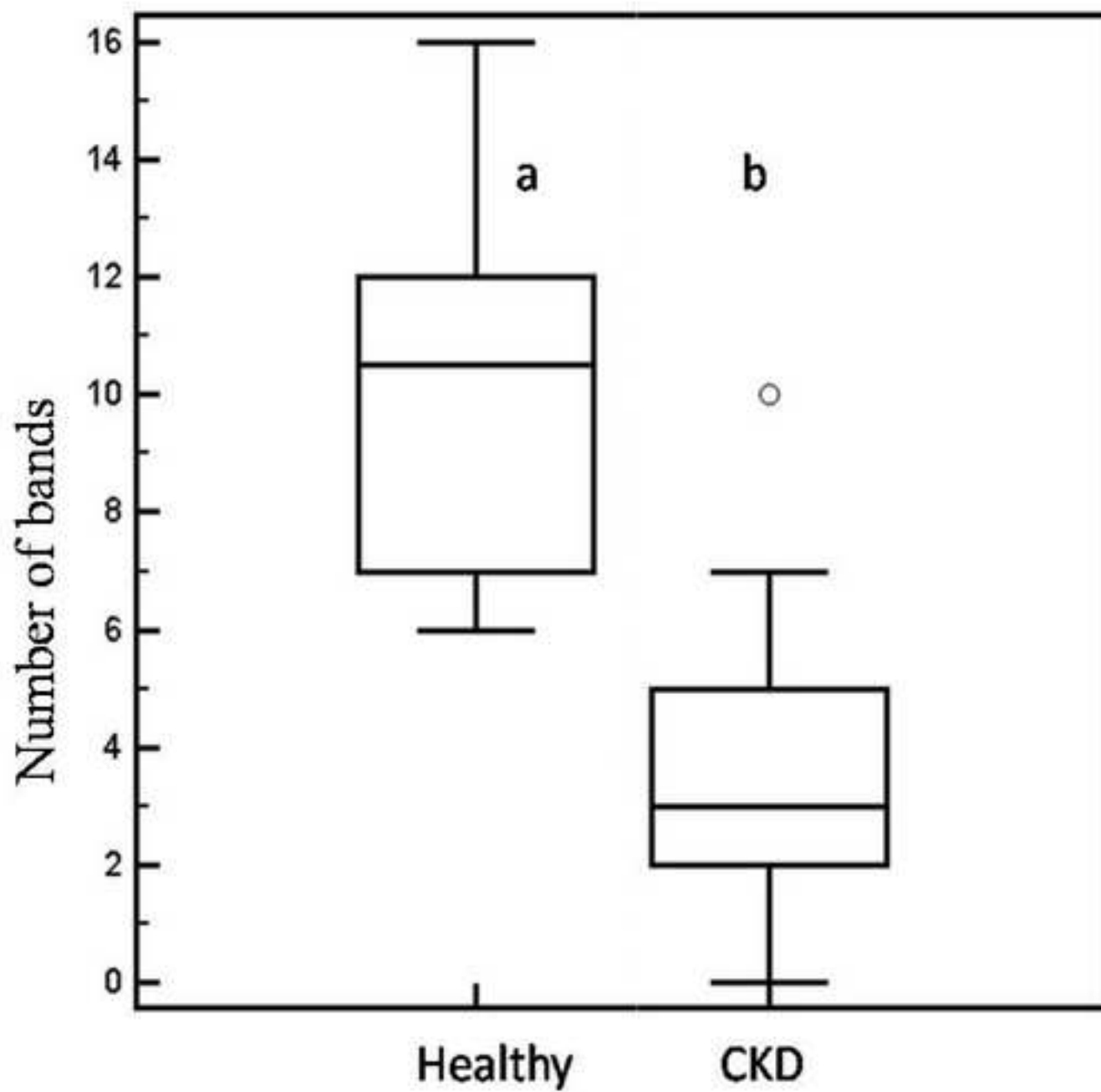
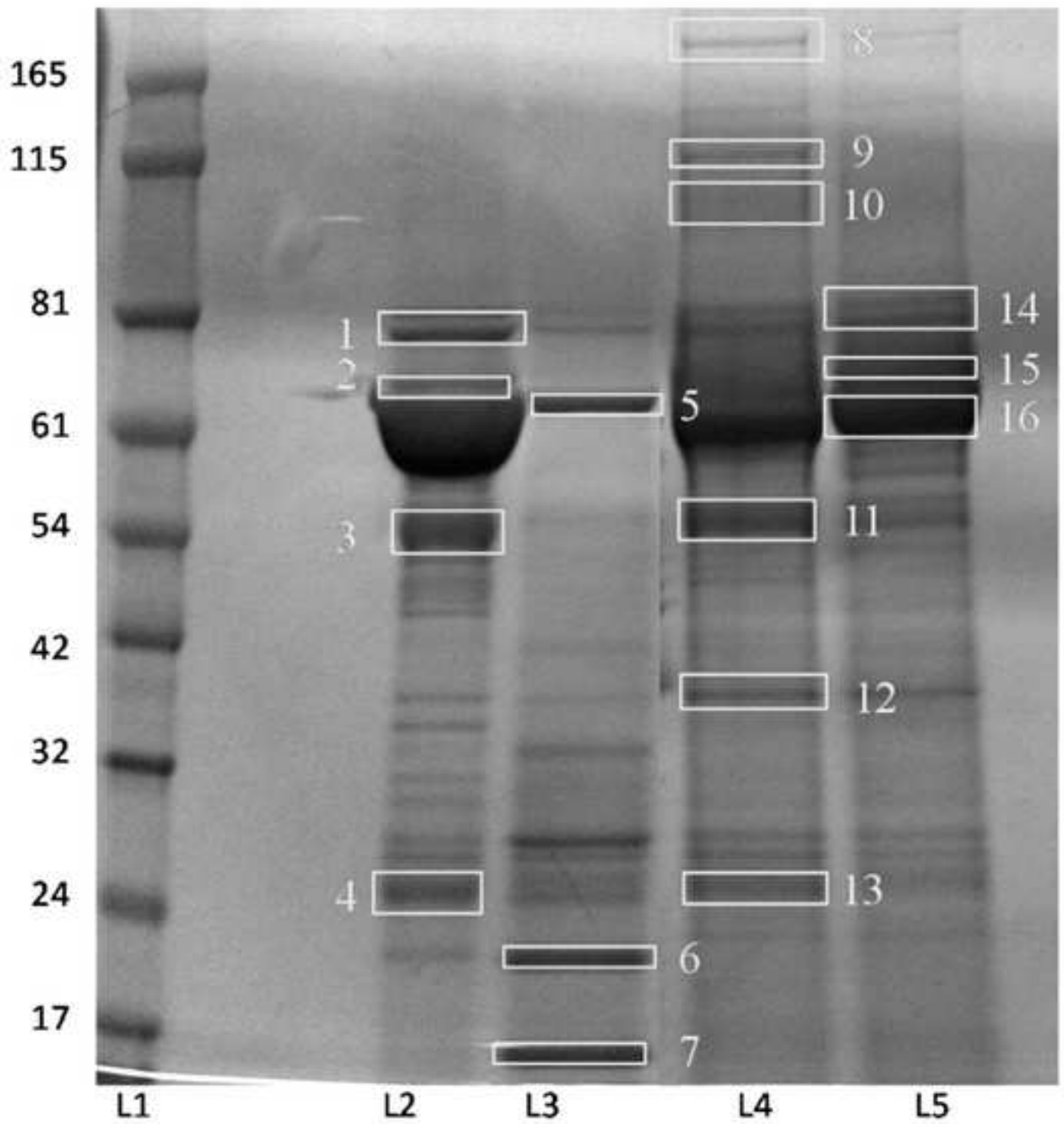


Figure3

kDa



A

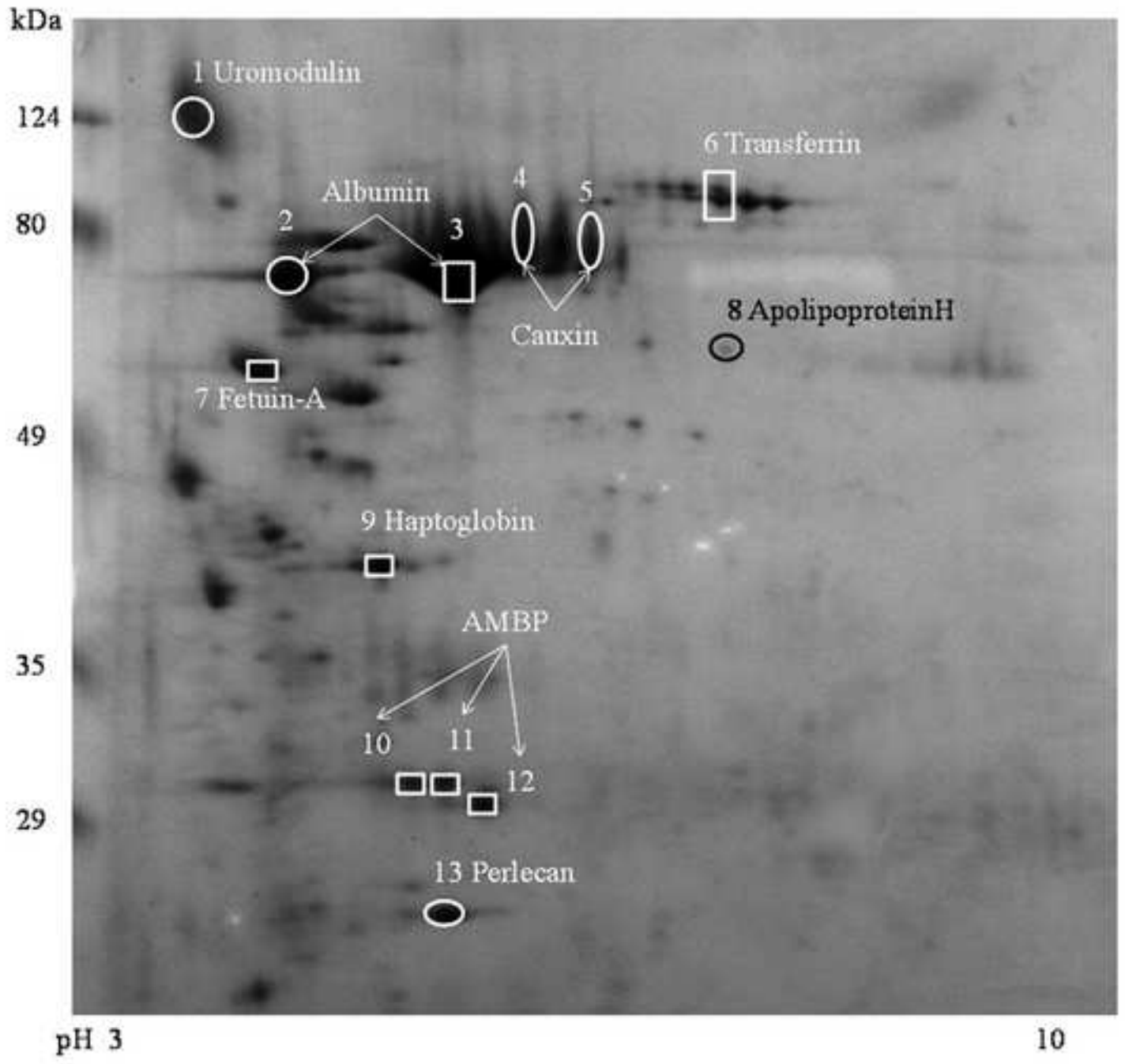
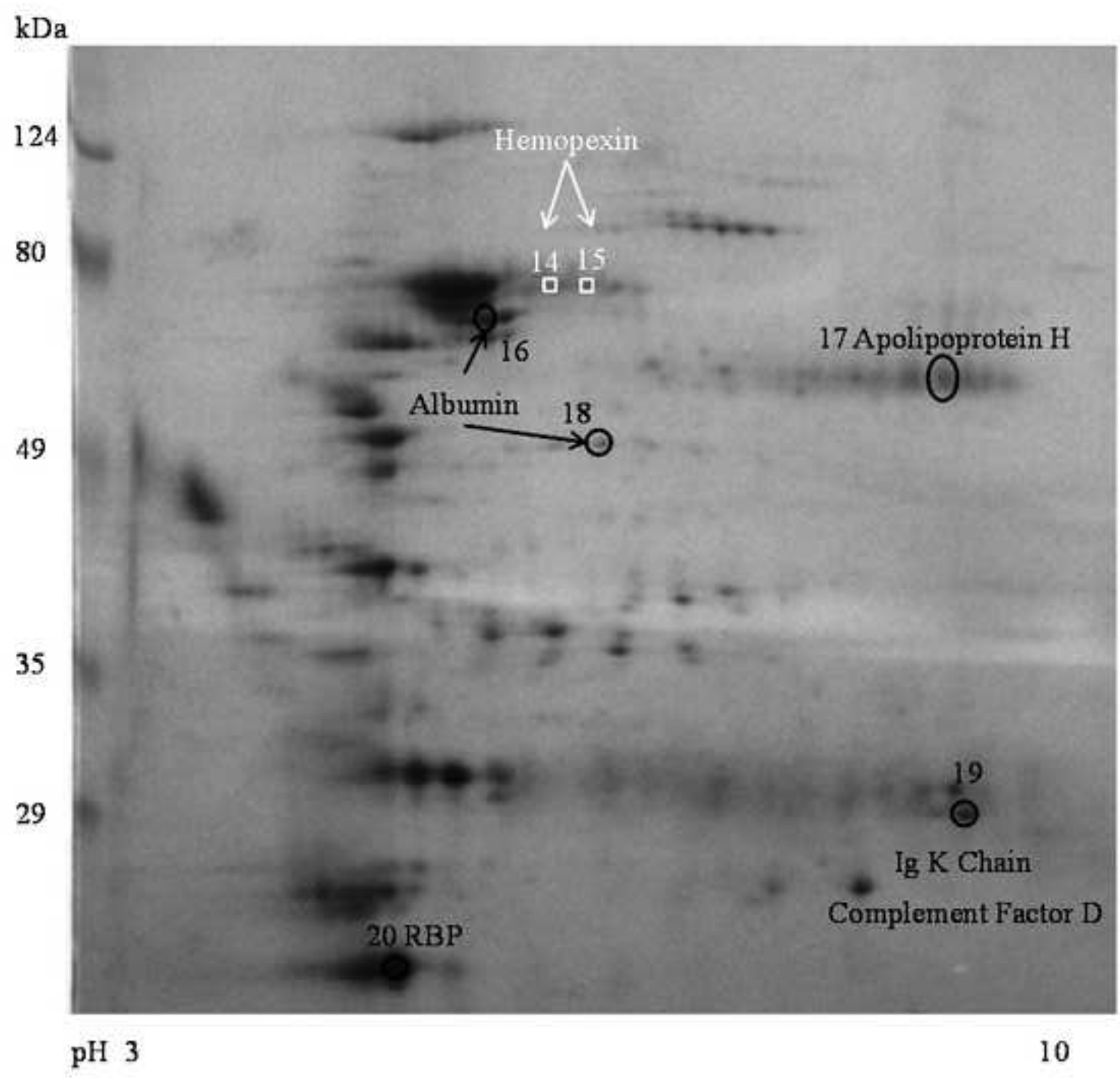


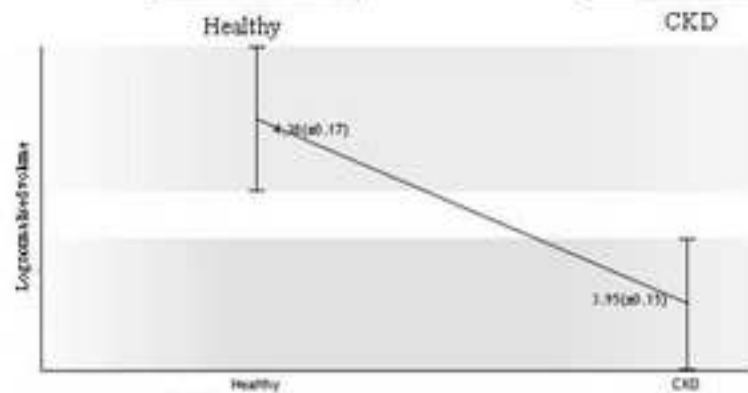
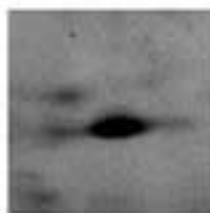
Figure4B



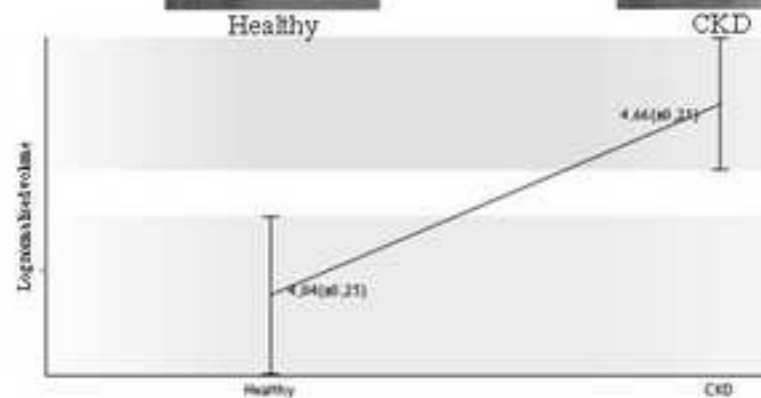
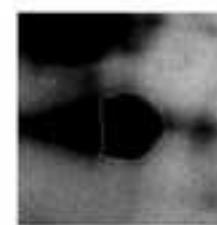
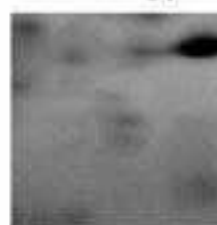


C

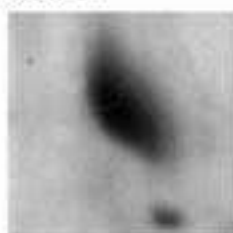
Spot 13: perlecan



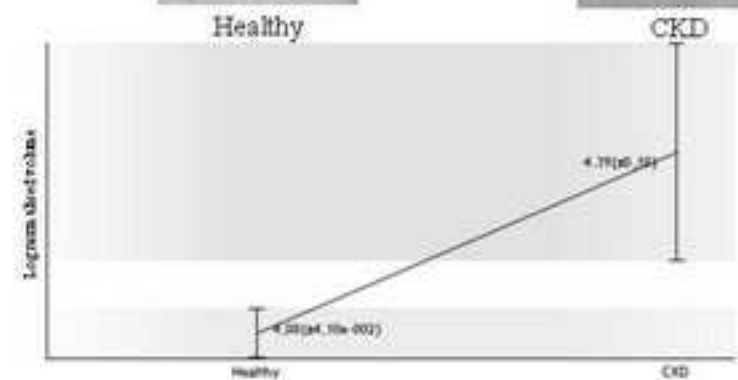
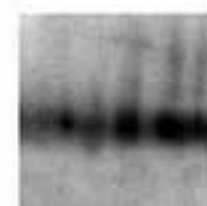
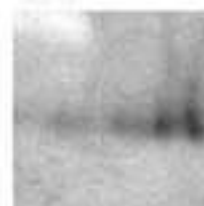
Spot 20: retinol binding protein



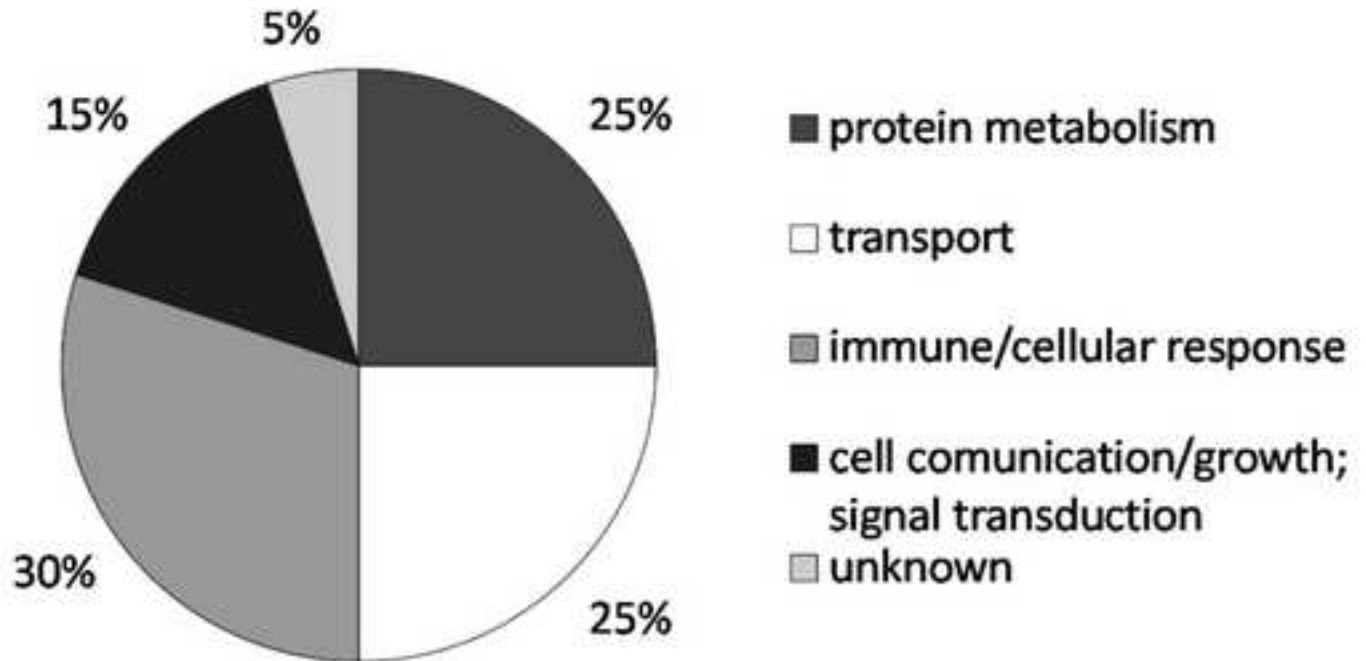
Spot 1: uromodulin



Spot 18: apolipoprotein-H



### Biological Process



### Localization

